

Supplementary Information

The Anti-tumor Toxin CD437 is a Direct Inhibitor of DNA Polymerase α

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Supplementary Results

Supplementary Table 1. Statistics of Exome-Sequencing.

Supplementary Dataset 1. Mutation results for exome sequencing of HCT-116 clones.

This is a list of all detected non-synonomous, splice site, and insertion/deletion mutations for 19 HCT-116 clones. 6 of these clones were resistant to CD437 (CD437R) and 13 others were sensitive (Control).

Supplementary Dataset 2. Genes with missense mutations in HCT-116 clones.

This is a list of genes for which exome sequencing revealed missense mutations in at least one of the 19 HCT-116 clones. Genes that harbored missense mutations in CD437R clones but not Control clones are highlighted and listed at the top.

Supplementary Figure 1. Target identification of cytotoxic compounds in HCT-116 cells.

Supplementary Figure 2. Sanger sequencing confirms *POLA1* L764S knock-in in HCT-116 and HeLa cells.

Supplementary Figure 3. Proteins and substrates for *in vitro* primer extension assay.

Supplementary Figure 4. Replicates of *in vitro* primer extension assay described in Figure 3C.

Supplementary Figure 5. Cell cycle analysis of HeLa cells subjected to thymidine block and release from thymidine block.

Supplementary Figure 6. Full gels are displayed for western blots shown in Figure 3E.

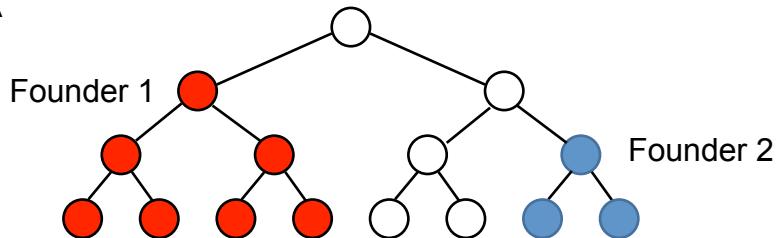
Supplementary Figure 7. Technical reproduction of CD437 and POLA1 binding studies described in Figure 4A and 4B.

Supplementary Table 1: Statistics of exome-sequencing

	CD437R_1	CD437R_2	CD437R_3	CD437R_4	CD437R_5	CD437R_6	Control_1	Control_2	Control_3	Control_4	Control_5	Control_6	Control_7	Control_8	Control_9	Control_10	Control_11	Control_12	Control_13
Total reads	88,658,868	81,254,624	66,807,460	95,204,150	107,545,996	142,755,820	88,973,384	282,819,720	295,430,916	311,601,212	284,239,330	275,059,304	272,751,324	277,506,698	296,382,804	282,401,946	271,827,274	316,441,436	288,154,752
QC-filtered reads	86,317,231	79,033,921	65,232,700	92,585,803	103,317,981	137,176,038	86,922,896	269,629,740	280,091,011	294,658,990	271,015,822	261,809,641	258,776,437	262,909,983	282,332,647	269,776,202	258,735,795	301,112,597	275,121,710
% QC-filtered reads	97.4%	97.3%	97.6%	97.2%	96.1%	96.1%	97.7%	95.3%	94.8%	94.6%	95.3%	95.2%	94.9%	94.7%	95.3%	95.5%	95.2%	95.5%	
mapped reads	85,500,997	78,126,200	64,549,137	91,452,175	102,689,398	136,079,976	86,108,813	262,549,175	272,588,917	286,705,282	263,743,716	254,695,730	251,987,275	256,129,467	274,724,970	262,326,485	251,978,622	293,959,139	267,827,920
% mapped reads	99.1%	98.9%	99.0%	98.8%	99.4%	99.2%	99.1%	97.4%	97.3%	97.3%	97.3%	97.3%	97.4%	97.4%	97.3%	97.2%	97.4%	97.3%	
non-duplicate reads	74,290,914	70,425,240	58,401,635	82,412,159	90,076,526	120,075,248	74,749,240	252,117,673	261,862,80	274,691,771	253,668,691	245,145,19	242,036,059	245,194,600	263,939,997	252,842,893	242,381,087	280,782,465	258,418,902
% non-duplicate reads	86.9%	90.1%	90.5%	90.1%	87.7%	88.2%	86.8%	96.0%	96.1%	95.8%	96.2%	96.3%	96.1%	95.7%	96.1%	96.4%	96.2%	95.5%	
on-target reads	63,890,040	60,979,674	50,509,862	71,071,220	78,074,724	104,636,040	65,166,466	103,362,015	101,149,072	104,041,020	103,913,443	100,118,599	100,471,468	99,208,040	102,170,500	98,494,919	95,690,818	114,236,079	98,275,324
% on-target reads	86.0%	86.6%	85.5%	86.2%	86.7%	87.1%	87.2%	41.0%	38.6%	37.9%	41.0%	40.8%	41.5%	40.5%	38.7%	39.0%	39.5%	40.7%	
mean depth	106.65	102.01	84.30	118.86	130.28	175.23	109.22	170.11	164.75	169.73	171.53	164.78	165.31	163.03	168.14	162.35	157.47	187.46	161.46
%_bases_above_1	99.8	99.8	99.8	99.8	99.8	99.8	100.0	100.0	99.9	100.0	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9
%_bases_above_5	99.6	99.5	99.4	99.6	99.6	99.6	99.6	99.8	99.8	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.8	99.7
%_bases_above_10	99.1	99.0	98.8	99.2	99.3	99.4	99.1	99.5	99.6	99.5	99.5	99.4	99.5	99.4	99.5	99.5	99.4	99.6	99.4
%_bases_above_20	97.6	97.3	96.1	97.9	98.3	98.7	97.6	98.7	98.8	98.8	98.7	98.6	98.7	98.6	98.7	98.6	98.9	98.7	98.7
Mean insert size	216.79	207.14	208.70	208.20	209.97	204.20	205.17	203.40	228.85	223.61	198.92	201.05	202.49	204.41	202.47	199.81	202.37	208.03	204.15
Standard deviation	68.82	60.82	59.57	61.08	58.74	57.52	59.50	58.19	74.78	72.92	58.50	56.92	57.15	57.74	59.84	57.22	57.88	57.78	58.35

Supplementary Figure 1

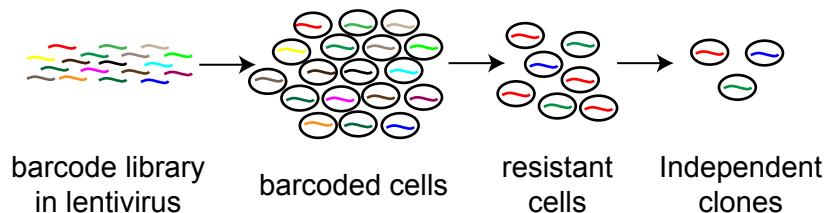
A



C

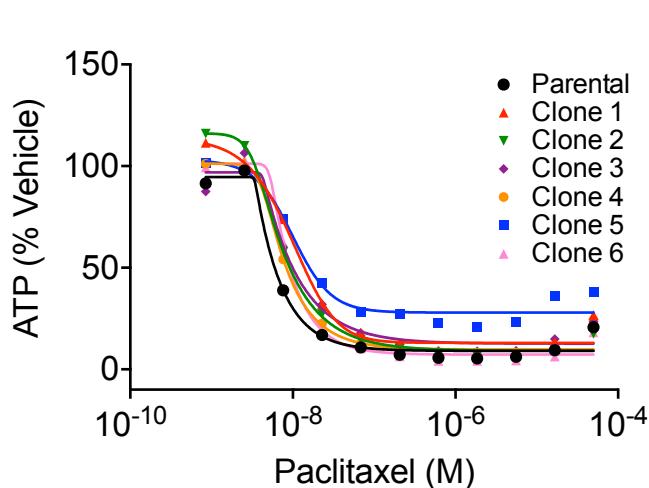
Clone ID	Barcode Sequence	Group	Exome -seq
19-1	GCCCCCGCCCGCCCCCGCG	A	1
19-2	GACCACNNNNNGCCNNGGG	B	
19-3	CCCGGGCCCNCCGTGTTNNGG	C	
19-4	CGCCCCNCGGNNCAGCAGNT	D	2
19-5	GAGGGGCAGGGGGGGCCCGGG	E	3
19-6	ANNGGNNCCNCCNTGTNNNNNG	I	
19-7	GNCGNCCCCCCCAGACCNGC	J	
19-8	no sequence		
19-9	GCCCCCGCCCGCCCCCGCG	A	
19-10	CNNNGCCCCCNCNNNCAGG	F	
19-11	CCNNNGCCCNCCTGTGTTNNGG	C	
19-12	no sequence		
19-13	no sequence		
19-14	GCCGCCCTCCCGCCCCCGGCC	G	4
19-15	CCCGCCCCCCCCNNGNCCGNG	H	
19-16	no sequence		
19-17	GCCCCCGCCCGCCCCCGCG	A	
19-18	CCCGGGCGGGCCCCCGCCCCGG	F	5
19-19	GCCCCCGCCCGCCCCCGCG	A	
19-20	ANNNNGCCCCCGTGTTCNNG	I	6

B



E

Clone #	IC50 (M)	
	CD437	Paclitaxel
Parental	3.06E-06	5.779E-09
1	3.18E-05	1.29E-08
2	1.54E-05	6.91E-09
3	1.92E-05	9.93E-09
4	1.12E-05	7.46E-09
5	1.73E-05	9.97E-09
6	1.81E-05	8.56E-09



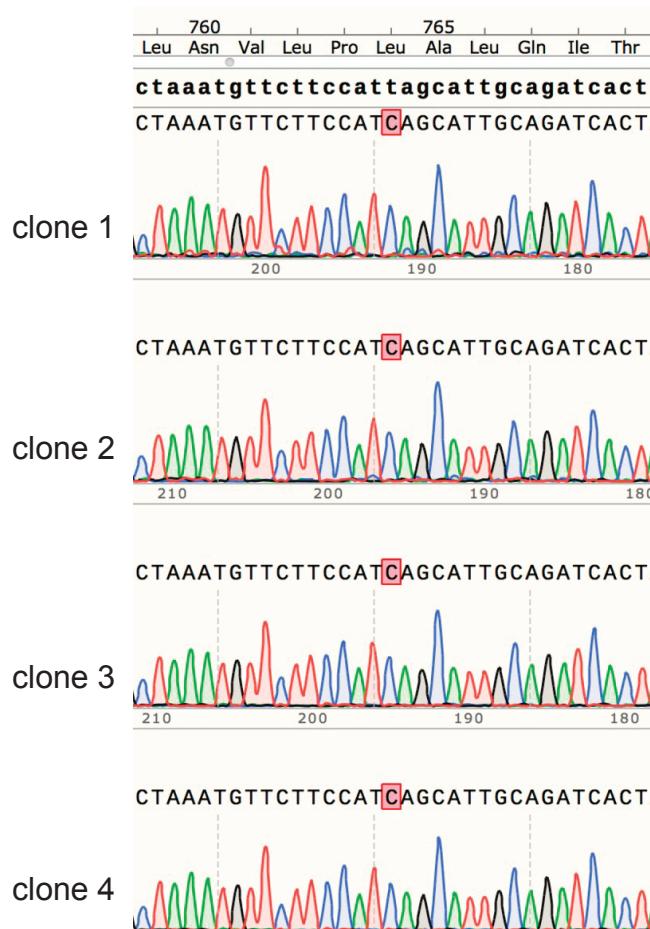
Supplementary Figure 1. Target identification of cytotoxic compounds in HCT-116 cells.

- (A) Resistant “founders” give rise to a family of resistant clones.
- (B) Efficient isolation of independent mutation events using a “barcoded” population of HCT-116 cells. Lines with different colors indicate different 20mer sequences.
- (C) Barcode sequences and groups.
- (D) Paclitaxel cross-resistance testing for CD437 resistant clones. Each point represents the average of two biological replicates.
- (E) IC50 values of CD437 and Paclitaxel for parental cells and CD437 resistant clones.

Supplementary Figure 2

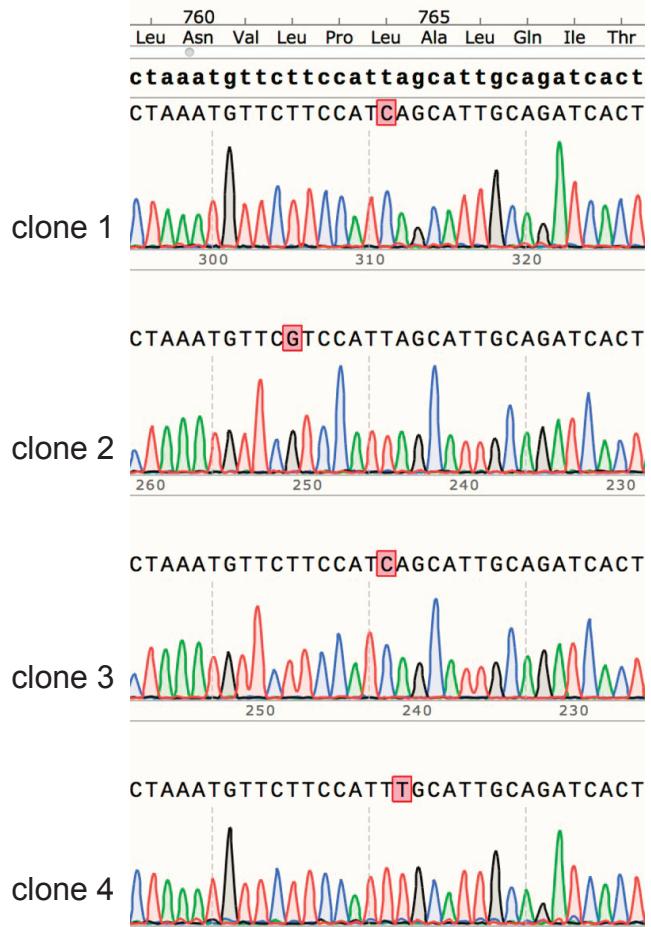
A

HCT-116 CRISPR clones



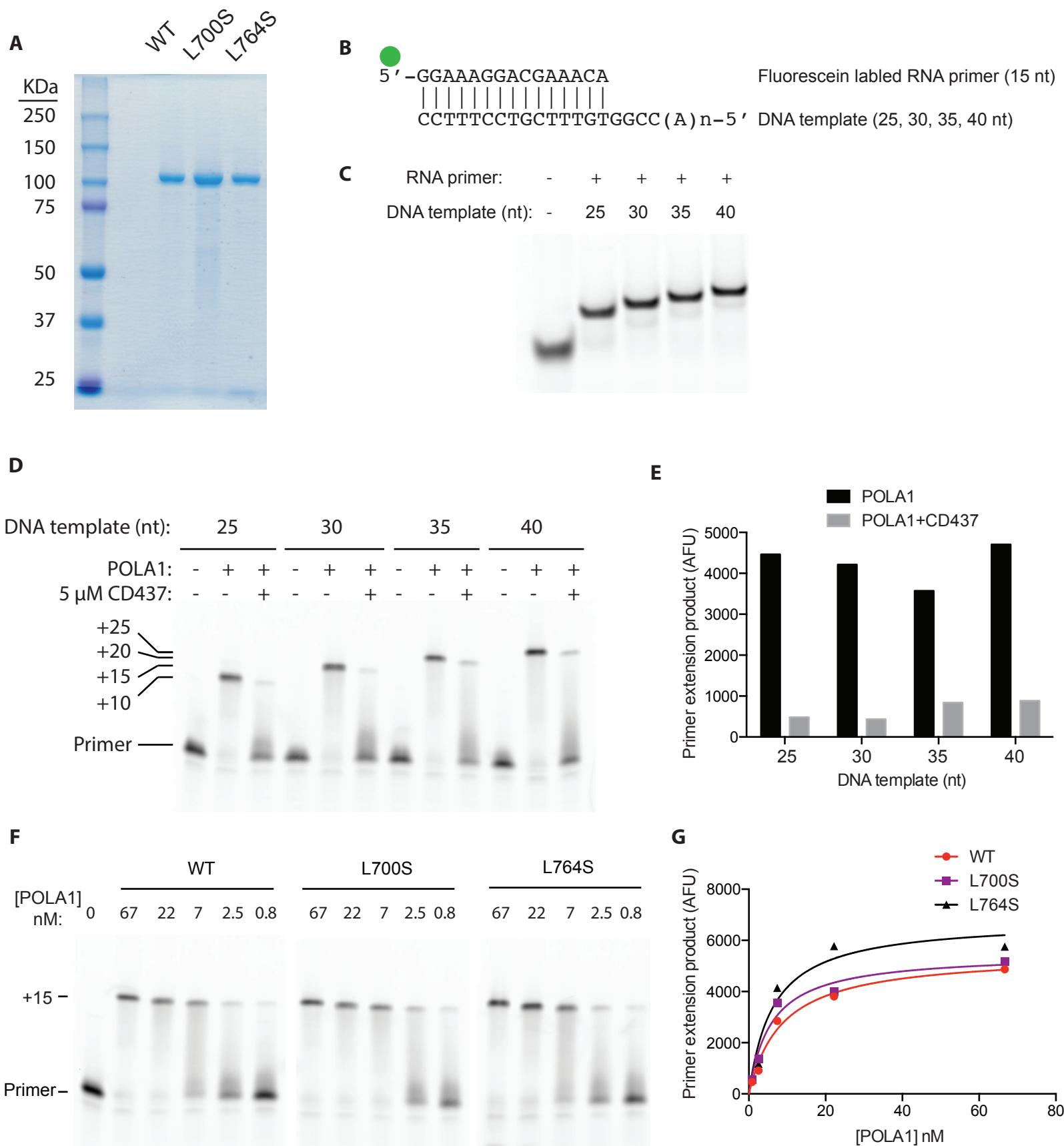
B

HeLa CRISPR clones



Supplementary Figure 2. Sanger sequencing confirms POLA1 L764S knock-in in HCT-116 and HeLa cells.

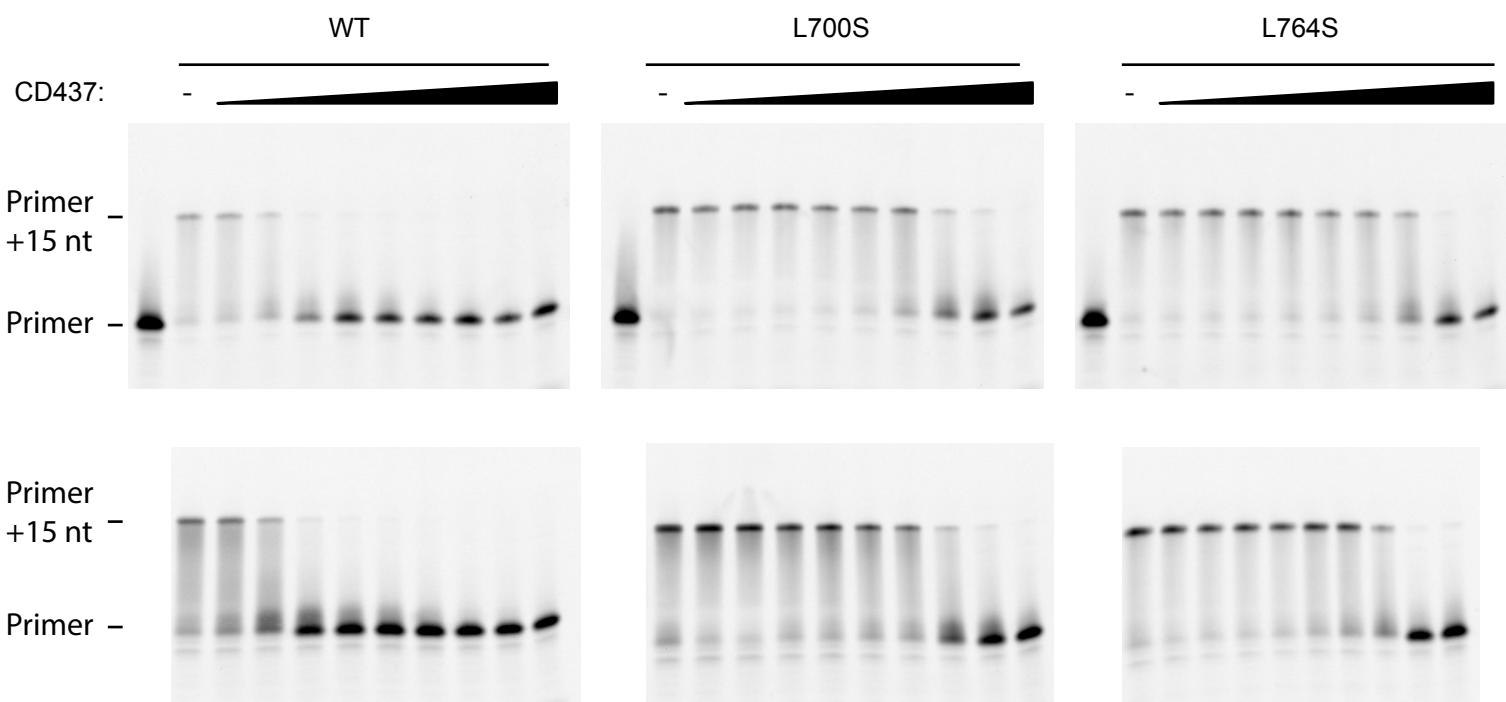
Supplementary Figure 3



Supplementary Figure 3. Proteins and substrates for in vitro primer extension assay.

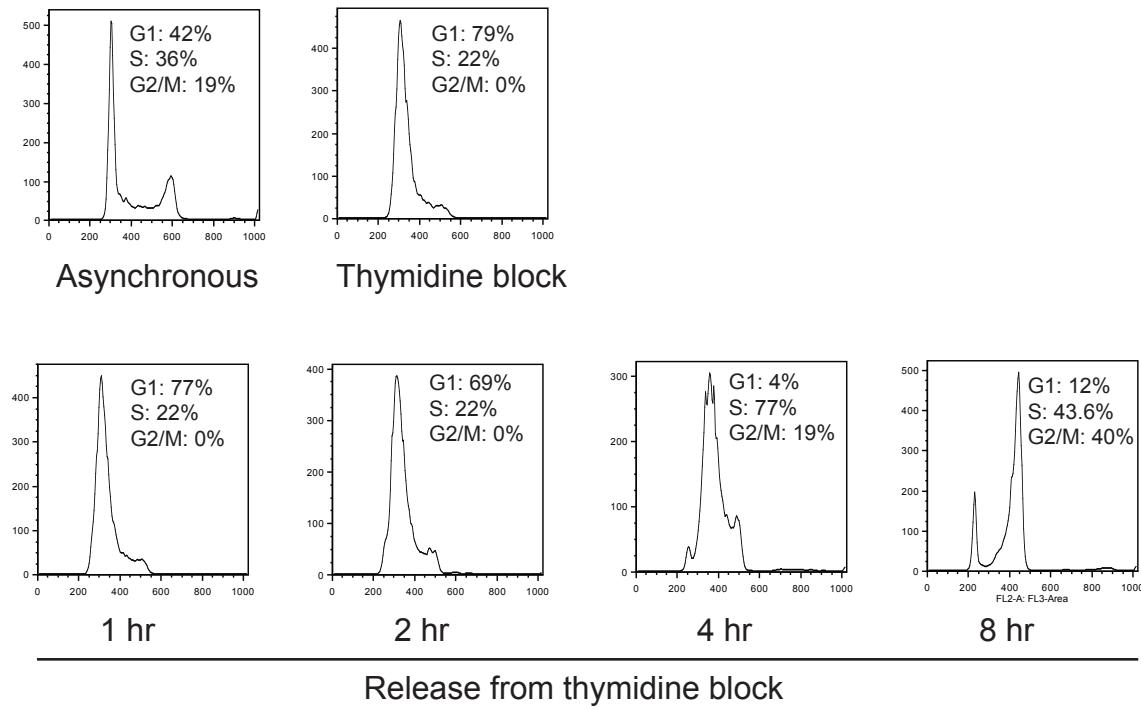
- (A) Coomassie stain of purified POLA1 proteins.
- (B) RNA-DNA duplexes used for in vitro primer extension assay.
- (C) Native gel electrophoresis conforms the formation of RNA-DNA duplexes.
- (D) Primer extension of different substrates by wild type POLA1. Incubation with 5 μ M CD437 inhibited POLA1 activity.
- (E) Quantification of primer extension product in (D). This experiment was performed once to optimize conditions for testing the effect of CD437 on in vitro activity.
- (F) The activity of varied concentrations of wild type and mutant POLA1 protein in primer extension.
- (G) Quantification of primer extension product in (F). These experiments were performed twice and a representative result is shown.

Supplementary Figure 4



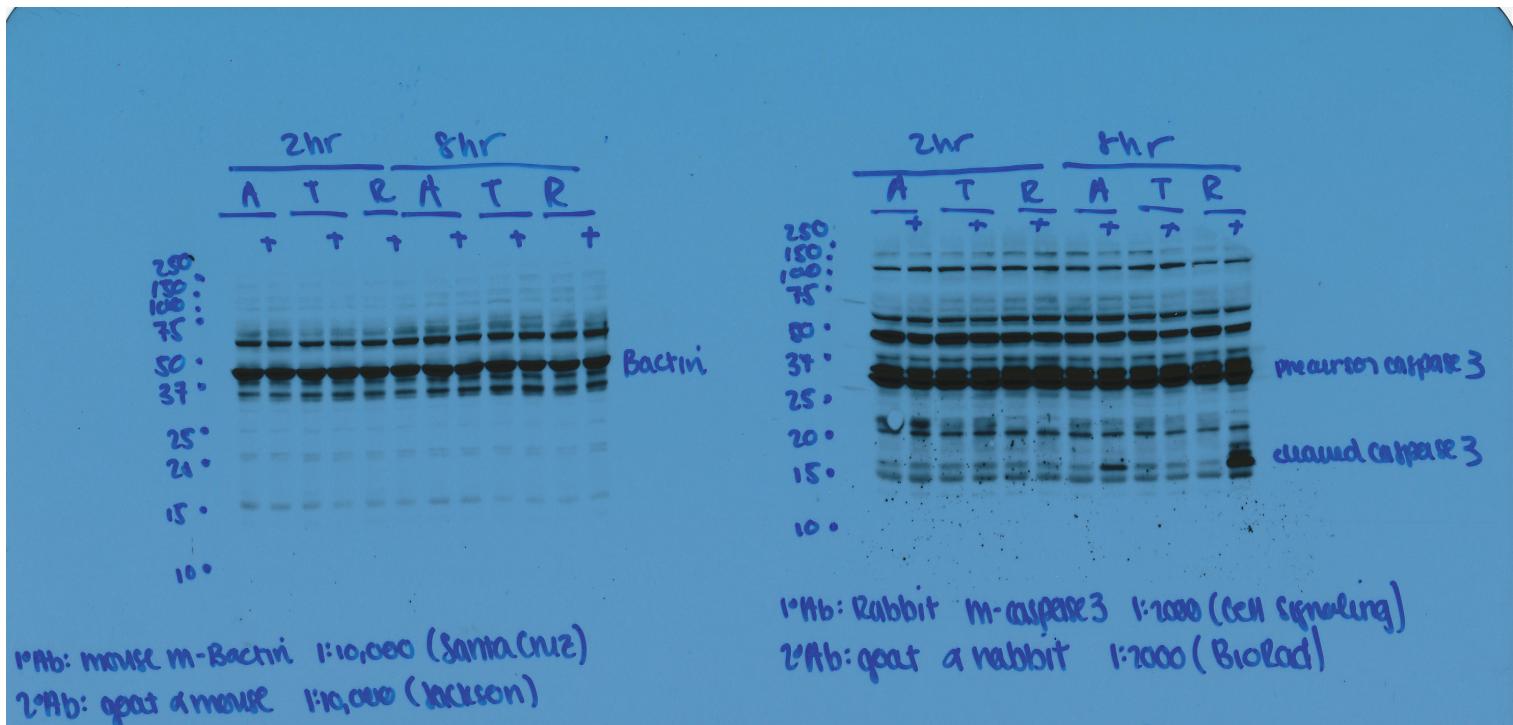
Supplementary Figure 4. Replicates of in vitro primer extension assay described in Figure 3C.

Supplementary Figure 5



Supplementary Figure 5. Cell cycle analysis of HeLa cells subjected to thymidine block and release from thymidine block. These experiments were performed twice and a representative result is shown.

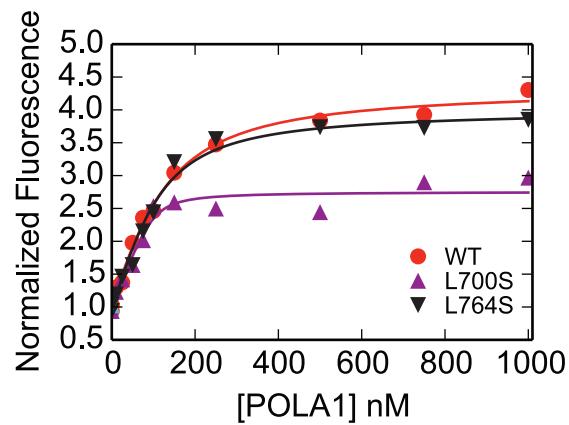
Supplementary Figure 6



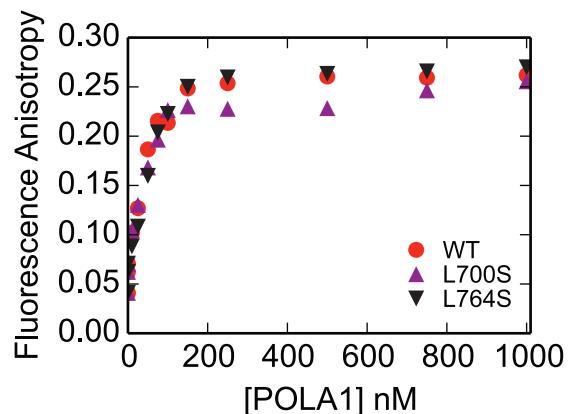
Supplementary Figure 6. Full gels are displayed for western blots shown in Figure 3E.

Supplementary Figure 7

A



B



Supplementary Figure 7. Technical reproduction of CD437 and POLA1 binding studies described in Figure 4A and 4B.